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ABSTRACT

Prostate cancer research is hampered by the lack of suitable human *in vitro* models that accurately recapitulate the genetic composition or biological behavior of initiate primary prostate tumors. In order to overcome this limitation, the N15C6 epithelial and the N1 fibroblastic cell lines were developed through immortalization of explanted human prostate tissue with the HPV E6 and E7 proteins. Genotypic characterization revealed that immortalization of the N15C6 epithelial cells was associated with a dominant genetic alteration involving the short arm of chromosome 8, a region often deleted or rearranged in primary human prostate tumors. Phenotypic characterization demonstrated that the N15C6 epithelial and N1 fibroblastic cell lines expressed cell-type appropriate proteins and gene transcripts. The N15C6 cells, but not N1 cells, expressed anchorage independence, consistent with an initially transformed phenotype. Moreover, N15C6 epithelial cells proliferated and formed colonies in soft agar at rates 2-3 fold higher when grown in N1 fibroblast conditioned serum free media compared to unconditioned serum free media. These results suggest that factors secreted by N1 fibroblasts augmented expression of the malignant phenotype by N15C6 epithelial cells, and show that paracrine interactions defined the malignant potential of transformed epithelial cells in the human prostate.

INTRODUCTION

Previous studies using reconstituted mouse urogenital sinus transformed through ectopic expression of activated proto-oncogenes (e.g., *myc* and *ras*) suggest that both epithelial and fibroblastic cell types are required for complete expression of the malignant phenotype by transformed epithelial cells (1, 2). Other studies, using human-rat tissue recombinants, also suggest that paracrine interactions between human prostate epithelial cells and rat urogenital sinus mesenchyme act homeostatically to maintain both cell types in a non-proliferative, differentiated state. This homeostasis is interrupted upon initial genetic damage to prostate epithelium, and continual, sequential disruption of epithelial-stromal homeostasis results in the dedifferentiation of emerging prostate carcinoma cells and surrounding stromal cells (3,4). Moreover, experiments utilizing co-cultured normal human prostate-derived fibroblasts and the LNCaP prostate metastasis-derived cell line clearly demonstrated that epithelial-stromal paracrine interactions were required for xenografted LNCaP cells to fully express the malignant phenotype and grow as tumors in athymic mice (5). Further work showed that fibroblasts associated with carcinoma stimulated tumor progression of immortalized non-tumorigenic epithelial cells *both in vitro* and *in vivo* (6).

Taken together, these studies show that epithelial-stromal paracrine interactions are likely required for human prostate tumorigenesis *in vivo*, and that this process may be initiated after primary genetic damage to epithelial cells. The application of these observations towards a detailed study of initial malignant transformation of human prostate epithelial cells, however, is hampered by the lack of suitable *in vitro* models. The 3 most intensively studied prostate-derived cell lines (PC3, DU145 and LNCaP) were established from metastatic lesions and possess highly aberrant karyotypes characterized by numerous structural and numerical chromosomal alterations (7,8). As such, it is unlikely that these cell lines accurately recapitulate the genetic composition or biological behavior of initiate human primary prostate tumors. In order to overcome these limitations, our laboratory attempted to develop a new, genetically and biologically accurate, *in vitro* model for early human prostate tumorigenesis comprising both epithelial and fibroblastic cell-type components. Here, we report the genotypic and phenotypic characterization of this model, and the use of this model to elucidate the contributions of epithelial-stromal paracrine interactions to early malignant transformation and maintenance of the transformed phenotype in the human prostate.

METHODS

Establishment of Cell Cultures. Normal prostate tissue samples were obtained aseptically from patients undergoing radical prostatectomy after cancer diagnosis. The tissue was minced into pieces <1 mm^2; one piece was fixed in 5% formalin for further histo-pathological evaluation, while the rest was digested overnight with 2mg/ml type 3 collagenase (Worthington Biochemical Corp. Lakewood, NJ) at 37°C with slow shaking (66 rpm). The digested tissue was passed through a small bore pipet and allowed to settle under gravity, washed twice, then plated into 60 mm collagen (Vitrogen 100, Cohesion)-coated plates in 5% HIEC medium [Ham's F12 (Mediatech Inc. Herndon, Virginia) with 5% FBS (Life Technologies, Inc.), 5 ug/ml insulin, 10 ng/ml EGF, 100 ug/ml cholera toxin and 1ug/ml hydrocortisone (Sigma chemical Co. St. Louis, MO) supplemented with antibiotics] (9).

Immortalization. Cells were immortalized through transduction with the recombinant LXSNE6E7 retrovirus harboring the HPV E6 and E7 genes as described previously (10). Transduced cells were selected using 400 ug/ml geneticin. After an initial round of cell death and crisis, cells resistant to geneticin grew out and were considered immortal after ten passages. Immortalized cell cultures were diluted 1:24 and individual subclones established.

Immunohistochemical Characterization. Cells were detached using trypsin and plated into sterile chamber slides (Fisher Scientific). Media was aspirated and the wells washed with 1X PBS. Cells were fixed using a 50% methanol/50% acetic acid solution for 15 minutes at 4°C, washed with 1X PBS, and stained using a pan cytokeratin monoclonal antibody mixture containing clones C-11, PRK-26, CY-90, KS-1A3, M20, and A53-B/A2 (Sigma) to detect epithelial cells, a monoclonal anti-vimentin antibody (Sigma) to detect stromal fibroblastic cells or a monoclonal antibody to von willebrand factor (VWF) (DAKO) to detect endothelial cells using the UniTect™ immunohistochemistry detection system (Oncogene Science Inc., Cambridge, MA) and manufacture's protocol. Slides were viewed using light microscopy. Subcloned cultures were considered homogenous when staining was apparent for only one cell type, e.g., vimentin for N1 fibroblastic cells and pan-cytokeratin for N15C6 epithelial cells. All subsequent experiments with these cell lines were performed at passage 23 for the N15C6 epithelial cells and passage 41 for the N1 fibroblastic cells.

Cell Proliferation. Cells were plated and counted in triplicate as described previously (11). Averages and standard deviations of cell number were calculated for each time point. The doubling time was calculated as the average number of hours required for the cell population to double in number.

Spectral Karyotype Analysis. Spectral karyotyping (SKY) analysis was performed as previously described (7,12). G-banding and SKY analyses were performed sequentially on each of the cell lines with the same ten metaphase images captured for G-banding also analyzed by SKY.

Soft Agar Colony Formation Assays. N15C6 cells were suspended in duplicate in 0.5% soft agar at densities of 10^5, 10^4, or 10^3 using previously described protocols (11). Serum-free ("SF") media was composed of Ham's F12 media supplemented to 5mM ethanolamine, 10mM HEPES, 5 ug/ul transferrin, 10 uM 3,3',5-triiodo-L-thyronine, 50 uM sodium selenite, 0.1% BSA, 100 units/ml penicillin and streptomycin, and 0.5 ug/ml fungizone. Each well was fed every three days with 1ml of one of the following media: SF media containing 1ug/ml hydrocortisone alone (SFH);); SF media containing 1ug/ml hydrocortisone + 10 ng/ml EGF (SFHE), SF media containing 1ug/ml hydrocortisone + 5 ug/ml insulin (SFHI), SF media containing 1ug/ml hydrocortisone + 5 ug/ml insulin + 10 ng/ml EGF (SFHIE), N1 fibroblast-conditioned serum-free media containing 1ug/ml hydrocortisone (SFH,CM), or N1 fibroblast-conditioned serum-free media containing 1ug/ml hydrocortisone + 5 ug/ml insulin + 10 ng/ml EGF (SFHIE, CM). The N1 fibroblast-conditioned serum-free media was prepared as follows: N1 cells were plated at passage 40, split once, washed with pre-warmed 1 X PBS, then fed with serum-free media containing either 1ug/ml hydrocortisone alone (SFH,CM) or 1ug/ml hydrocortisone + 5 ug/ml insulin + 10 ng/ml EGF (SFHIE, CM). After 24 hours the SFH,CM or SFHIE,CM media was removed from the N1 cell cultures and fed to the N15C6 cells embedded in soft agar. All cultures were fed with fresh media every 2-3 days. After three weeks, cells were stained by overnight incubation with 10ug/ml tetrazolium violet (Sigma Chemical Co.) in the media at 37°C with CO_2. Colonies ere counted using an AccuCount apparatus (Biologics, Inc.). HT-29 cells were used as positive controls for colony formation.

Androgen Sensitivity Assays. 50,000 N15C6 cells per assay were plated into sterile six-well plates in triplicate and grown for seven days in SF or SF,CM media supplemented with 10^{-7}, 10^{-8}, or 10^{-9}M methytrienolone (R1881) (NEN, Inc.) (11). Cells were lysed and nuclei counted as described above.

RNA Profiling by cDNA Microarray. N1 cells were grown in SFHIE and N15C6 cells were grown in either SFHIE or SFHIE,CM media for 24 hours, washed with 1XPBS, and lysed using Trizol reagent (Life Technologies, Inc.) to prepare RNA following manufacturers instructions. The RNA was further purified using RNeasy reagents (Qiagen), and 20 ug of total RNA from each sample was labeled with Cy5 or Cy3 fluorescent dye using CyScribe first strand cDNA labeling kit. After purification on AutoSeq G50 columns (Amersham Pharmacia Biotech UK), the labeled probes were combined, dried down to 5 ul, and added to 45 ul SlideHyb #1 hybridization buffer (Ambion Inc. Houston TX) pre-warmed to 68°C. The probe/hybridization mixture was pre-incubated at 68°C for 5 minutes, then transferred to the cDNA microarray slide which was coverslipped and incubated in a moisture chamber at 45°0C for 16 hours. The slides were washed for 10 minutes each in 2X SSC, 0.2% SDS at 45°C, 2X SSC at room temperature, and 0.2X SSC at room temperature, rinsed, dried, and evaluated using a GenePix 4000A Axon Laser Scanner and associated software. Each experiment was performed twice with the dyes "switched" in the second experiment to assess data reproducibility. All experiments utilized either the 1.6K or 4.1K Human Cancer Arrays comprising cDNAs for genes implicated in tumorigenesis (a complete list of the genes on both arrays can be obtained at http://www.umich.edu/~caparray). Designations for cell type-specific expression of the genes on the array were derived from the National Center for Biotechnology UniGene (http://www.ncbi.nlm.nih.gov/UniGene) and SAGE (Serial Analysis of Gene Expression) databases (http://www.ncbi.nlm.nih.gov/SAGE), and the GeneCards database at the Weizmann Institute of Science (http://genecards.ym.edu.tw).

Statistical Analysis. cDNA microarray data was first normalized within each array using rank-invariant normalization followed by quantile normalization using source code described at http://www.stat.lsa.umich.edu/~kshedden/Normalize. After normalization, data points were considered for further analysis if their average log intensities were greater than 8, corresponding to an average fluorescence intensity of 256. Data points with an average log intensity of less than 8 were excluded because these values were close to background and were considered less reliable. Genes were then classified as up-regulated if both of the following conditions were met: 1) The $\log_2(Cy5/Cy3)$ was greater than 0.5 when RNA from N15C6 cells grown under conditioned media was labeled with Cy5 and under serum-free media was labeled with Cy3, and 2) the $\log_2(Cy5/Cy3)$ was less than -0.5 when RNA from N15C6 cells grown under conditioned media was labeled with Cy3 and under serum-free media was labeled with Cy5. Genes were considered down-regulated if the opposite conditions were met.

RESULTS

At the 10th passage of cell culture, N15 epithelial cells were considered immortal and were removed from geneticin selection. To establish a homogeneous culture, N15 epithelial cells were then diluted 1:24 and the resulting 24 subclones were characterized immunohistochemcially for the expression of epithelial- or fibroblast-specific proteins. Consistent with an epithelial cell type, the majority of cells from all 24 subclones expressed high levels of cytokeratins. In particular, the C6 subclone expressed cytokeratins but not vimentin proteins, consistent with a homogeneous epithelial cell type without fibroblast contamination (Figure 1). This C6 subclone cell line, referred to as N15C6, was used for all subsequent experiments. The stromal tissue-derived N1 cells expressed vimentin exclusively, consistent with a homogenous fibroblastic cell type (Figure 1). Cell proliferation assays established doubling times of 57 hours for the N15C6 epithelial cells and 32 hours for the N1 fibroblastic cells. RNA profiling assays using spotted arrays comprising cDNAs for 1644 human genes demonstrated expression of 1131 genes by either N1 or N15C6 cells. Of these, 47 (12%) transcripts were differentially expressed between the two cell lines by a factor of $\geq$3-fold, with 16 gene transcripts preferentially expressed (upregulated) in N15C6 epithlial cells and 31 gene transcripts preferentially expressed in N1 fibroblastic cells (Table I). Cell-type specificity was epithelial for the majority (12/15, 75%) of transcripts expressed preferentially by N15C6 cells, and genes specifically expressed by N15C6 epithelial cells included four cytokeratin genes (KRT5, KRT7, KRT8 and KRT 19). None of the genes preferentially expressed by N15C6 cells were described as specific to stromal tissues or fibroblastic cell types. Of the 31 genes preferentially expressed by N1 cells, 10 (32%) have been described as stromal-specific, 2 (7%) as epithelial-specific, 17 (55%) as expressed by both stromal and epithelial tissues, and 2 (7%) as expressed by other tissue types (Table I). Three collagenase genes, COL1A2, COL6A1 and COL6A1, were exclusively expressed by the N1 fibroblast cells. Thus, the RNA profiles for the N1 and N15C6 cells were largely consistent with cell-type.

Because human papillomavirus E6 and E7-mediated immortalization can induce chromosomal aberrations (13-15), both the N15C6 and N1 cells were genotypically characterized using spectral karyotyping. The karyotype for the N15C6 epithelial cell line was determined as 42~44, X, der(Y)dupinv(q11q12), der(8)t(8;19)(q10;p10), +der(20)t(8;20)(p11;q10)x2, -19, -21, -21, -22. Structural aberrations involving the pericentromeric (p10 and q10) region of chromosome 8 were the most frequently observed chromosomal defect in N15C6 cells (Figure 1). The N1 fibroblastic cells exhibited a normal human diploid 46 X,Y karyotype (not shown).

The N15 cells had undergone genetic modification as a result of HPV E6/E7-mediated immortalization. Therefore, experiments were conducted to determine whether the cells had also undergone malignant transformation. Characterization of the ability of the N15C6 or N1 cell lines to express anchorage independence, a hallmark of the malignant phenotype, was assessed by their ability to grow as colonies in soft agar. When grown in serum-free media (SF) supplemented with either hydrocortisone alone (SFH), hydrocortisone and EGF (SFHE), or hydrocortisone and insulin (SFHI), neither the N15C6 epithelial nor N1 fibroblast cells were anchorage independent (Figure 2). However, the ability of N15C6 cells to grow as colonies in soft agar was augmented by a factor of 2-3 fold by growth in serum-free media supplemented with hydrocortisone, insulin and EGF (SFHIE) ($p<.003$) (Figure 2). Moreover, growth in N1-conditioned SFHIE media further augmented expression of anchorage independence by N15C6 cells an additional 2-3 fold ($p<.0001$). In contrast, the presence of one or more of the growth factors insulin, hydrocortisone or EGF did not enhance the ability of N1 cells to become anchorage independent.

Because androgen is a major growth factor for prostate epithelium, the N15C6 cells were examined to determine whether they would respond proliferatively to synthetic androgen. N15C6 cells demonstrated a modest proliferative response when grown in SF media supplemented with 10^{-7}M, 10^{-8}M or 10^{-9}M R1881 compared to growth in SF media alone (Figure 3). However, N15C6 cells exhibited a pronounced proliferative response when grown in SF,CM media and achieved cell numbers 2.5X higher compared to growth in SF media alone ($p<.001$). This augmented proliferative response was entirely ablated by the addition of 10-^{7}M R1881. Exposure to successively lower doses of R1881 resulted in restoration of the proliferative effects of the SF,CM media (Figure 3).

The observation of a pronounced proliferative response and augmented expression of anchorage independence by N15C6 cells when grown in N1 fibroblast-conditioned media was further investigated at the genetic level through RNA profiling experiments using spotted arrays comprising cDNAs for 4170 human genes. After normalization, a group of 18 genes (Table II) were identified that were differentially expressed by the N15C6 epithelial cells after exposure to N1 fibroblast-conditioned media (Table II).

DISCUSSION

Prostate cancer research is hampered by the lack of suitable *in vitro* models that accurately recapitulate the genetic composition or biological behavior of initiate human primary prostate tumors. In order to overcome these limitations, our laboratory developed two new cell lines from human prostate tissue, the N15C6 epithelial and the N1 fibroblastic cell lines, through immortalization of explanted human prostate tissue with the HPV E6 and E7 proteins.

Karyotypic characterization showed that the dominant genetic alteration observed for N15C6 epithelial cells involved chromosomes 20 and 8. Alterations, especially duplication, of chromosome 20 or the 20q region alone are observed in many cancer types and are clearly associated with immortalization of human cells (16). Moreover, alterations of chromosome 8, especially those involving 8p, may comprise the most frequent and "earliest" genetic change during human prostate tumorigenesis (17, 18). Because alterations involving chromosomes 20 and 8 are associated with immortalization and early malignant transformation in the prostate, the observation of these alterations in N15C6 cells is consistent with the characterization of these cells as representative of nascent malignantly transformed prostatic epithelium.

Immunohistochemical and RNA profiling experiments demonstrated that the N15C6 epithelial and N1 fibroblastic cell lines expressed specific proteins and transcriptional profiles largely consistent with their cell type. In the presence of minimal growth factors, the N15C6 epithelial, but not the N1 fibroblastic, cells were able to express anchorage independence. Finally N15C6 cells demonstrated a modest proliferative response to physiological levels of synthetic androgen. These results suggest that the N1 fibroblastic cell line is immortalized but not transformed whereas the N15C6 epithelial cell line is immortalized and initially transformed. Furthermore, the N1 cells were genotypically normal whereas the N15C6 cells were genotypically similar to premalignant and early malignant lesions in the human prostate. These results are consistent with those expected for a suitable model for elucidation of the contributions of epithelial-stromal paracrine interactions to early malignant transformation and maintenance of the transformed phenotype in the human prostate.

Exposure to N1 fibroblast-conditioned media resulted in augmented expression of the malignant phenotype by N15C6 cells. N15C6 cells proliferated and formed colonies in soft agar at rates 2-3 fold higher when grown in N1 fibroblast conditioned serum free media compared to unconditioned serum free media. These results suggest that factors secreted by N1 fibroblasts augmented the malignant potential of N15C6 epithelial cells, and are consistent with an important role for paracrine interactions in malignant transformation in the human prostate.

The observed augmented proliferation of N15C6 epithelial cells in response to N1 fibroblast conditioned media was reduced by the addition of synthetic androgen to the culture media. This suggests that factors secreted by the N1 fibroblasts may be involved in direct or indirect interactions with the androgen axis in N15C6 cells. Further studies are clearly required to elucidate potential androgen/fibroblast factor interactions regulating N15C6 cellular proliferation.

Consistent with the observed changes in phenotypic expression, N15C6 epithelial cells demonstrated changes in genotypic expression at the transcriptional level in response to factors secreted by N1 fibroblasts. Eighteen genes were identified that were differentially expressed by N15C6 epithelial cells after exposure to N1 fibroblast conditioned media. Two of these genes, KIAA0618 and DKFZp761B2423, encode hypothetical proteins. The KIAA0618 ORF encodes a protein with homology to nuclear envelope pore membrane proteins. However, the DKFZp761B2423 gene encodes a hypothetical protein with no known functional motifs (http://pfam.wustl.edu).

Among the genes upregulated after exposure to conditioned media were lipocalcin 2, LOC64116, and STK39. All three genes encode proteins potentially involved in enhanced cellular survival and prostate-specific metabolism. Lipocalcin 2/uterolcalcin is transcriptionally activated in tumors of the breast, colon and pancreas and induces apopotosis of infiltrating neutrophils, protecting cells from the degradative enzymes, free radicals and other secreted products of activated phagotcytes (19). Pfam analysis (http://pfam.wustl.edu) demonstrated that the LOC64116 gene encodes a protein with a ZIP, or zinc transporter, domain. Interestingly, PC3 and LNCaP prostate cancer cells upregulate expression of a zinc transporter protein in response to hormonal stimulation, suggesting that one or more proteins secreted by fibroblasts may act to upregulate expression of the LOC64116 zinc transporter protein in N15C6 cells (20). The STK39 gene encodes serine-threonine kinase 39, a protein that can activate the p38 pathway, is induced by androgens in LNCaP cells, and may act as a mediator of stress-activated signals (21).

Downregulated genes included caveolin 1, caveolin 2, amphiregulin and thrombospondin. The caveolins are downregulated in many tumor types, and re-expression of caveolin-1 in colon or breast cancer cells is associated with reduced tumorigenicity (22, 23). Interestingly, suppression of caveolin expression induces androgen sensitivity in androgen insensitive, metastatic mouse prostate cancer cells and caveolin levels are actually elevated in metastatic and hormone refractory prostate tumors. These data suggest that N15C6 cells express a phenotype consistent with that of a hormone sensitive primary tumor, and that this phenotype remains more similar to a primary rather than metastatic tumor after exposure to fibroblast-conditioned media. Amphiregulin is an EGFR ligand and its expression is abundant in prostatic smooth muscle cells but heterogeneous in benign prostatic hyperplasia and prostate tumors (24). Thrombospondin 1 protein expression is downregulated in both primary and metastatic prostate tumors (25, 26). It is a potent inhibitor of angiogenesis, suggesting that its downregulation may be permissive for angiogenesis and tumor growth.

Several genes involved in cell-cell adhesion were differentially expressed by N15C6 cells after exposure to fibroblast conditioned media. Upregulated cell-cell adhesion genes included CD44, cadherin 3, and claudin 1. The CD44 gene is expressed primarily by basal epithelial cells in the prostate, which are believed to give rise to secretory luminal cells. De novo expression of CD44 has been associated with systemic dissemination of prostate cancer, though decreased CD44 expression has also been associated with metastatic disease (27, 28). Cadherin 3 protein upregulation has been associated with high cellular proliferation rates, has been implicated as an early step in malignant transformation in the breast and colon, and is an independent prognostic predictor for poor survival in breast carcinomas (29 - 31). Upregulation of claudin 1 gene expression has also been observed in colorectal cancers, and other members of the claudin family (3, 4, 7) are upregulated in breast and ovarian cancers (32 - 34). Interestingly, claudin 1 transcription is regulated by β-catenin, suggesting that exposure to fibroblast conditioned media may alter β-catenin mediated transcription in N15C6 cells (34). Cathepsin L, another cell-cell adhesion gene, was downregulated by N15C6 cells after exposure to N1 fibroblast conditioned media. An inverse correlation between cathepsin L protein expression and tumor grade has been observed in ovarian carcinoma, suggesting that cathepsin L may be downregulated during tumor progression (35). Taken together, these changes in gene expression induced by exposure to N1 fiboblast conditioned media resulted in the production of proteins appropriate for a primary prostate tumor implicated in enhanced the survival and growth.

In summary, we report the creation of a suitable human *in vitro* model for elucidation of the contributions of epithelial-stromal paracrine interactions to early malignant transformation and maintenance of the transformed phenotype in the prostate. Experiments utilizing this model demonstrate that human transformed prostate epithelial cells and prostate fibroblast cells participate in paracrine interactions. These paracrine interactions clearly result in augmented expression of the malignant phenotype and correlative, possibly causative, changes in gene expression by transformed epithelial cells. Use of this *in vitro* model and suitable *in vivo* models should define how paracrine-driven changes in the expression levels of specific genes augments and defines expression of the malignant phenotype in the human prostate.

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FIGURE LEGENDS

Figure 1. Immunohistochemical and Karyotypic Characterization. A. N15C6 epithelial and N1 fibroblast cells stained with hematoxylin/eosin (H/E) or immunohistochemically evaluated for vimentin or cytokeratin expression (magnification = 400X). **B.** Spectral karyotype composite of alterations involving chromosomes 8 and 20 in N15C6 epithelial cells.

Figure 2. Anchorage Independence. A. Photomicrographs showing colony formation in soft agar for N1 fibroblast cells and N15C6 epithelial cells in specific media (magnification = 100X). **B**. Histogram of soft agar colony densities for N1 fibroblast (grey bars) and N15C6 epithelial (black bars) cells grown in the indicated medias. SF = serum free media; H = hydrocorticose; E = EGF; I = insulin; CM = N1 fibroblast conditioned media.

Figure 3. Sensitivity of N15C6 Epithelial Cells to Androgen Stimulation. Histogram of proliferative response of N15C6 epithlial cells to increasing concentrations of the synthetic androgen R1881 in the absence (black bars) or presence (grey bars) of N1 fibroblast-conditioned media. SF = serum-free media; CM = N1 fibroblast conditioned media.

Figure 4. Northern Blot Validation of RNA Profiling of N15C6 Epithelial Cells Grown in Serum-Free versus Serum-Free N1 Fibroblast Conditioned Media. Almost done (needs GAPDH on two blots) for lipocalcin 2, LOC64116, amphiregulin and caveolin 2.

Figure 1

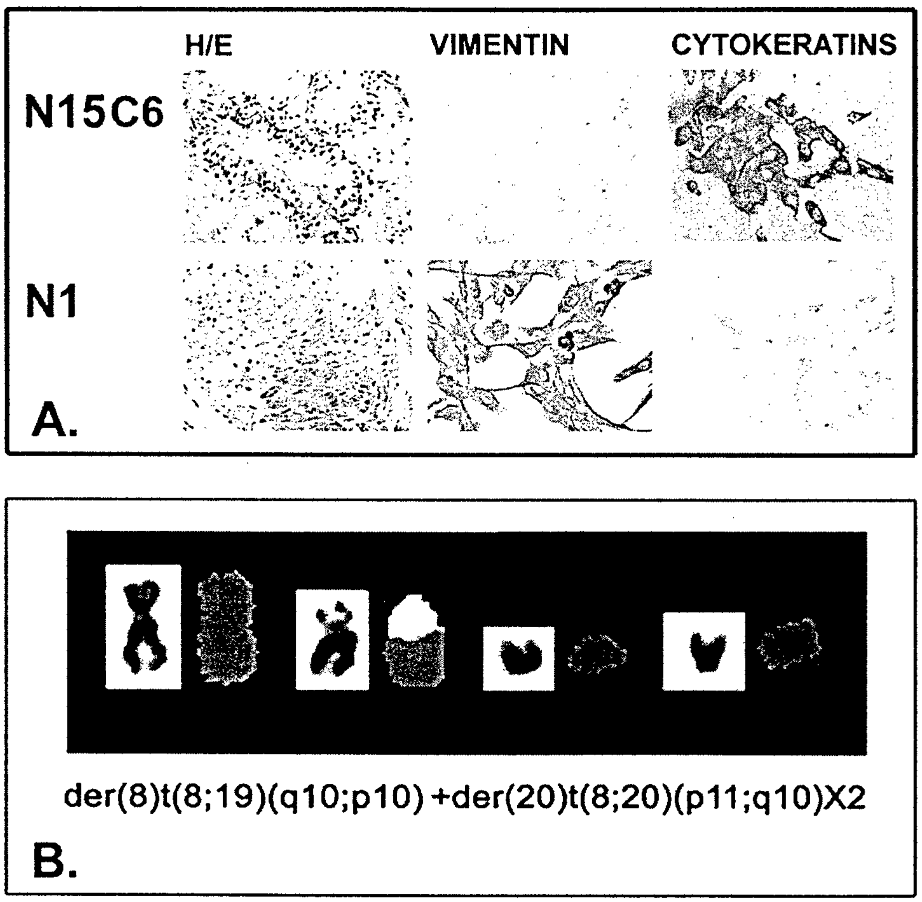

H/E
VIMENTIN
CYTOKERATINS
N15C6
N1
A.
der(8)t(8;19)(q10;p10) +der(20)t(8;20)(p11;q10)X2
B.

Figure 2

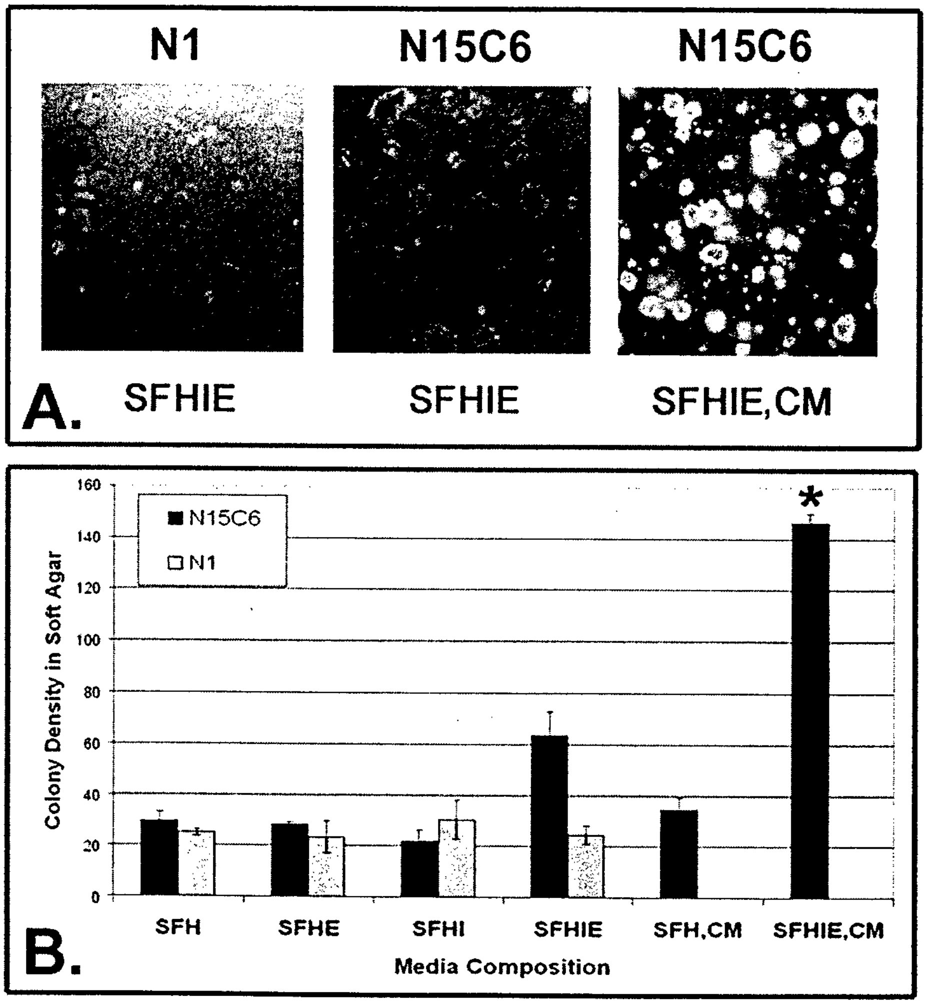
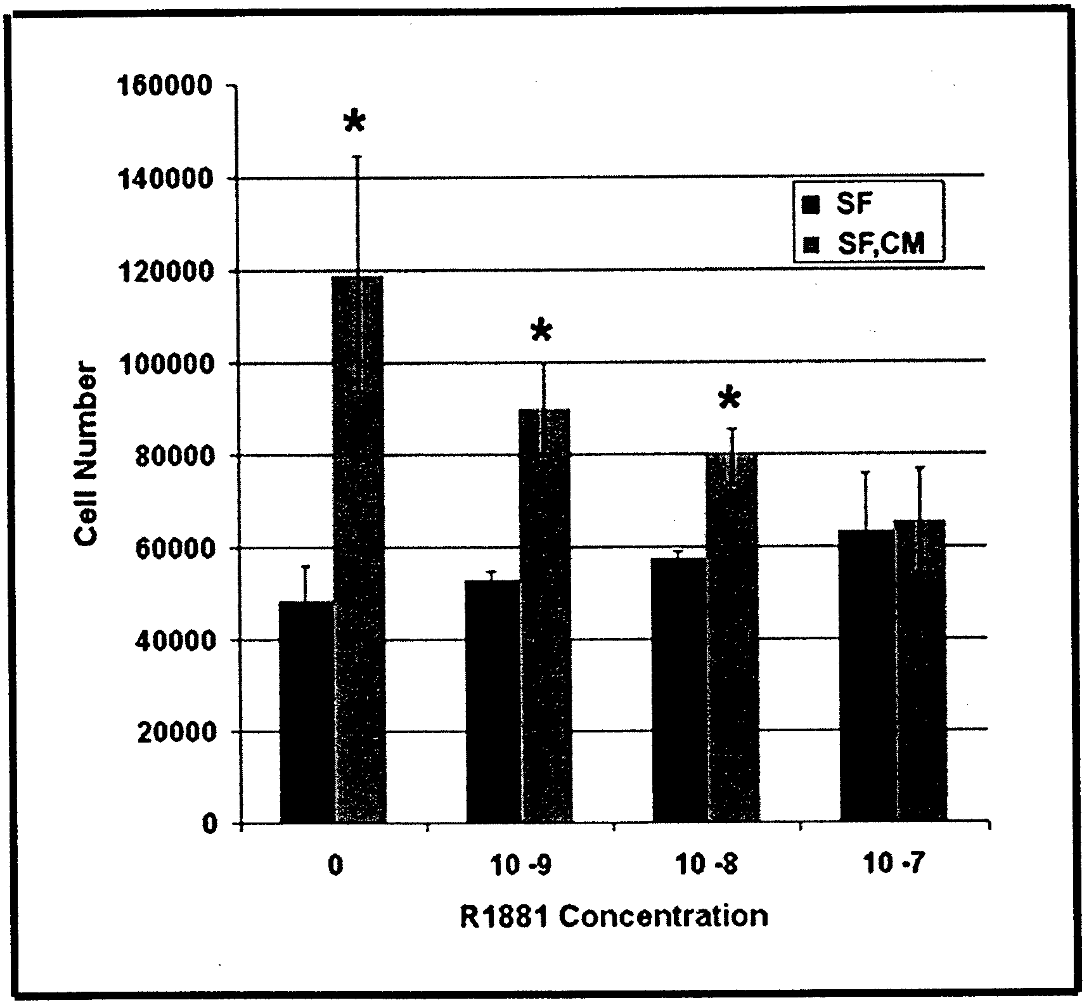

N1
N15C6
N15C6
A.
SFHIE
SFHIE
SFHIE,CM
160
140
120
100
80
60
40
20
0
N15C6
N1
*
Colony Density in Soft Agar
SFH
SFHE
SFHI
SFHIE
SFH,CM
SFHIE,CM
B.
Media Composition

Figure 3



160000
140000
120000
100000
80000
60000
40000
20000
0
Cell Number
SF
SF,CM
*
*
*
0
10 -9
10 -8
10 -7
R1881 Concentration

TABLE I

RNA Expression Profiling of N1 Fibroblastic and N15C6 Epithelial Cells

UPREGULATED IN N1 FIBROBLASTIC CELLS

Gene	N15 vs N1	N1 vs N15	Log Difference	Expression Pattern	Cell Type Specificity
STIP1	-4.605	5.067	9.672	Up In N1	S
MMP2	-3.923	4.432	8.355	Up In N1	S
UBL1	-2.794	3.913	6.707	Up In N1	S,E
COL1A2	-1.844	4.029	5.873	Up In N1	S
DAF	-3.149	2.672	5.821	Up In N1	S,E
CD44	-3.036	2.770	5.806	Up In N1	S,E
INPPL1	-2.397	2.482	4.879	Up In N1	S,E
COL6A3	-2.153	2.489	4.642	Up In N1	S
BMP7	-2.432	2.141	4.573	Up In N1	E
IGHM	-2.459	2.018	4.477	Up In N1	S,E
FTH1	-3.048	1.416	4.464	Up In N1	S,E
RFC2	-1.955	2.479	4.434	Up In N1	S,E
FLJ20701	-1.785	2.647	4.432	Up In N1	S,E
CTGF	-2.091	2.339	4.430	Up In N1	S
COL6A1	-1.978	1.845	3.823	Up In N1	S
PLAUR	-1.506	2.317	3.823	Up In N1	S,E
ADAMTS1	-1.973	1.768	3.741	Up In N1	S,E
SRPX	-1.541	2.143	3.684	Up In N1	S,E
FN1	-2.034	1.647	3.681	Up In N1	S
GAS6	-1.593	1.858	3.451	Up In N1	S
PFKM	-1.575	1.722	3.297	Up In N1	S,E
EST	-1.81	1.395	3.205	Up In N1	brain
LRP2	-1.711	1.439	3.150	Up In N1	E
CRI1	-1.788	1.267	3.055	Up In N1	S
C4BPA	-1.558	1.463	3.021	Up In N1	S,E
USP8	-1.87	0.955	2.825	Up In N1	S,E
FGFR1	-1.794	1.015	2.809	Up In N1	S
RPL18A	-1.626	1.154	2.780	Up In N1	S,E
EEF1A1	-1.637	0.933	2.570	Up In N1	S,E
PPAP2B	-1.596	0.870	2.466	Up In N1	S,E
ENC1	-1.505	0.815	2.320	Up In N1	brain

UPREGULATED IN N15C6 EPITHELIAL CELLS

Gene	N15 vs N1	N1 vs N15	Log Difference	Expression Pattern	Cell Type Specificity
KRT5	4.017	-3.237	7.254	Up in N15C6	E
FOSL2	5.850	-1.069	6.919	Up in N15C6	E
MAP7	2.396	-4.073	6.469	Up in N15C6	E
KRT7	2.638	-3.667	6.305	Up in N15C6	E
KRT19	1.740	-4.000	5.740	Up in N15C6	E
KRT8	1.820	-2.539	4.359	Up in N15C6	E
PPARBP	2.188	-1.975	4.163	Up in N15C6	S,E
IFIT1	1.934	-2.116	4.050	Up in N15C6	E
GSTP1	1.958	-2.038	3.996	Up in N15C6	E
LAMC2	1.728	-2.226	3.954	Up in N15C6	E
ITGA7	2.585	-1.037	3.622	Up in N15C6	E
PTPN2	2.386	-0.929	3.315	Up in N15C6	S,E
LRP1	1.540	-0.990	2.530	Up in N15C6	E
UGT2B17	1.524	-0.959	2.483	Up in N15C6	E
C1orf29	1.663	-0.718	2.381	Up in N15C6	S,E
MYC	1.509	-0.829	2.338	Up in N15C6	S,E

Note: In Cell Type Specificity, S=stromal and E=epithelial

Table II

Effect of N1 Fibroblast Conditioned Media on N15C6 Epithelial Cell Gene Transcription

Gene	GenBank	CM vs SF	SF vs CM	Log Difference	Effect of CM
lipocalin 2 (oncogene 24p3)	AA401137	-1.923	1.634	3.56	Up
up-regulated by BCG-CWS LOC 64116	AA521384	-1.077	1.291	2.37	Up
cadherin 3	AA425556	-1.230	1.031	2.26	Up
IFI16 interferon gamma-inducible protein 16	AA491191	-1.227	0.553	1.78	Up
metallothionein 1X STK9 serene-threonine kinase 39	N80129	-0.831	0.794	1.63	Up
Macrophage receptor with collagenous structure	AA485867	-0.507	1.110	1.62	Up
claudin 1	AA194833	-0.824	0.791	1.61	Up
GS2NA nuclear autoantigen	AA418918	-0.651	0.836	1.49	Up
KIAA0618 gene product	H81940	-0.955	0.523	1.48	Up
CD44 antigen	AA282906	-0.611	0.721	1.33	Up
amphiregulin	AA857163	1.094	-1.421	2.50	Down
caveolin 2	T89391	1.360	-1.045	2.40	Down
thrombospondin 1	AA464630	0.642	-1.247	1.90	Down
hypothetical protein DKFZp761B2423	R88741	0.985	-0.700	1.70	Down
cathepsin L	W73874	1.056	-0.658	1.70	Down
MYL6 Myosin, light polypeptide 6	AA488346	1.125	-0.517	1.60	Down
DnaJ (Hsp40) homolog, subfamily B	AA435948	0.879	-0.686	1.60	Down
caveolin 1	AA055835	0.943	-0.665	1.60	Down

tion of new prostate specific genes could provide new markers and could be instrumental for development of new treatment modalities. Tensin is an actin-binding protein that contains a Src homology 2 (SH2) domain and phosphotyrosine-binding (PTB) domain. Tensin knockout mice developed renal disease and showed defects in wound healing. During the course of isolating tensin family genes, we have identified a novel C-terminal tensin-like molecule, cten. Cten is a 715 amino acid molecule containing SH2 and PTB domains that are similar to tensin. However, cten does not have the actin-binding and focal adhesion-binding activities found in tensin family. Northern blot analysis showed that the expression of cten is restricted to prostate and placenta, suggesting that cten plays a critical role in these tissues. To explore the potential role of cten in prostate cancer, we have examined the mRNA levels of cten in prostate cancer/ cell lines. The initial studies showed that cten expression is often reduced/lost in cancer samples. These results suggest that alteration of cten expression may disrupt its normal function and increases the risk for prostate cancer. To examine cten protein localization, we generated a GFP-cten construct and transfected it into prostate cells. In contrast to the focal adhesion localization of GFP-tensin molecules, the majority of GFP-cten was found in the cytoplasm. Furthermore, when the N-terminal half of cten was deleted, the remaining portion of cten was found predominantly in nuclei, whereas GFP alone was found in the cytoplasm. These findings suggest that cten might be a signal transducer traveling from the cytoplasm to the nucleus.

#5220 p21 is involved in H-cadherin regulated contact inhibition of cell growth. Yun Zhong, Lluis Lopez, Missak Haigentz, Yihe Ling, and Roman Perez-Soler. *Department of Oncology, Albert Einstein College of Medicine, Bronx, NY.*

H-cadherin, unlike other classic cadherins such as E-cadherin, N-cadherin and P-cadherin, lacks a cytoplasmic domain. Abnormalities in the H-cadherin gene have been identified in a variety of human cancers. Recent evidence indicates that H-cadherin expression inhibits tumor cell invasiveness *in vitro* and tumor formation *in vivo*. However, the role of H-cadherin in regulating cell growth is unknown. We have studied the role of H-cadherin cell-cell adhesion and cellular proliferation in Chinese hamster overy(CHO)cells by transfection of the human H-cadherin cDNA. By phase contrast microscopy, exogenous H-cadherin expressing CHO cells were more round and had less spindle morphology compared with the control CHO cells. Plating efficiency assays showed that the colonies from the H-cadherin expressing CHO cells were several fold smaller than those from control CHO cells. H-cadherin expressing CHO cells had reduced proliferation rates at high cell density in a contact inhibtion growth assay. The lower proliferation rate of H-cadherin expressing cells was associated with decreased cell shedding. A cell cycle profile analysis demonstrated that the H-cadherin expressing cells had a higher percentage of cells at G1 phase compared with the control CHO cells. Interestingly, seeding H-cadherin expressing CHO cells on culture dishes coated with a recombinant H-cadherin amino-terminal fragment inhibited their proliferation. Meanwhile, the recombinant H-cadherin protein induced G1 arrest of H-cadherin expressing cells and was accompanied by elevation of p21 protein levels. Taken together, these data demonstrate that H-cadherin functions as a tumor suppressor gene that is involved in contact inhibition of cell growth by inducing cell cycle arrest at G1 phase accompanied with increased p21 expression. This work was supported in part by NIH Grants CA86845 and CA84119.

#5221 Paracrine interactions modulate phenotypic and genotypic expression in transformed prostatic epithelial cells. Rajiv Kant, Mark A. Rubin, Ben Beheshti, Jeremy A. Squire, Johng Rhim, and Jill A. Macoska. *The University of Michigan, Ann Arbor, MI, The University of Toronto, Toronto, ON, Canada, and Uniformed Services University of the Health Sciences, Rockville, MD.*

Alterations in cellular phenotype and gene activity associated with acquisition and progression of the malignant phenotype in the human prostate may be further modulated through paracrine interactions. In order to test this hypothesis and examine the effects of paracrine interactions on the malignant transformation of human prostate epithelial cells, we have created an *in vitro* model system for nascent prostate tumorigenesis comprising HPV E6/E7- immortalized, genetically characterized prostate epithelial and fibroblast cell lines. Spectral karyotyping demonstrated structural rearrangements involving chromosomes 8, 19 and 20, and losses of chromosomes 19, 21 and 22 in the prostate epithelial cells, but no chromosomal aberrations in the fibroblast cells. Growth of the transformed prostate epithelial cells in serum-free fibroblast-conditioned media enhanced expression of the malignant phenotype, observed as an increased expression of anchorage independence. cDNA microarrays were utilized to compare the transcription profiles of over 1000 cancer-related genes in transformed prostate epithelial cells grown in serum-free media or serum-free fibroblast-conditioned media. RNA profiling demonstrated that paracrine interactions clearly modulated the transcriptional activity of specific genes including some encoding tumor suppressors (p53, Rb, DAB2, SH3BP2), oncogenes (AKT1), proteins involved in cell proliferation (PPP2R5E, PCTK3, PPBP), oxidative stress (NCF1), and the immune response (TNFRSF5, IFNAR1) that may facilitate tumorigenesis in the human prostate. These experiments demonstrate that a novel *in vitro* model system successfully mimics fibroblast-epithelial paracrine interactions in the human prostate. Moreover, such paracrine interactions clearly modulate the transcriptional activity of specific genes that may facilitate prostate tumorigenesis. Further studies are aimed at understanding the contribution of these differentially expressed gene products towards nascent tumorigenesis in the human prostate.

#5222 Alterations in catenins due to MMP-mediated cleavage of E-cadherin in polarized epithelial cells. Barbara Fingleton and Lynn M. Matrisian. *Vanderbilt University, Nashville, TN.*

Loss of function of the homophilic cell:cell adhesion molecule E-cadherin is associated with tumor progression, invasion and metastasis. One way this can be achieved is through proteolytic cleavage and we have previously shown that the matrix metalloproteinases MMP-3 (stromelysin-1) and MMP-7 (matrilysin) can cleave E-cadherin releasing the 80kDa ectodomain [1]. The soluble ectodomain can then destabilize further E-cadherin binding interactions resulting in loss of cell adhesion and increased invasive ability of targeted cells. The aim of our current study is to determine if MMP-mediated cleavage of E-cadherin alters cellular signaling pathways. Catenins, proteins that associate with the cytoplasmic tail of E-cadherin at adherens junctions, are known to interact with a number of signaling pathways. In epithelial E-cadherin-positive cells treated with MMP-7, localization of the catenin p120 changes from membrane-associated to cytosolic. This is postulated to alter signaling via the small G protein rho. Exposure to MMP-7 also results in increased phosphorylation of β−catenin and an apparent cleavage of gamma catenin (plakoglobin). Treatment of polarized epithelial cells with MMP-7 results in loss of polarization and increased proliferation. These effects are not observed when the enzyme is added to the apical surface of cells where E-cadherin is not found. Our results suggest that changes in cell signaling molecules due to the activity of an MMP can contribute to the tumorigenic properties of cells and thus expand the potential roles for these proteases in the tumor progression process. [1] Noe, V. et al (2001) Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. J. Cell Sci. 114, 111-118.

#5223 Discoidin domain receptor phosphorylation kinetics: Inhibitory effect of adhesion. Corine G. M. L'Hote, Peter H. Thomas, and Trivadi S. Ganesan. *Institute of Molecular Medicine, Oxford, UK.*

Discoidin Domain Receptor 1 (DDR1), a receptor tyrosine kinase (RTK) has been shown to be activated mainly by soluble fibrillar collagen. Unusually, the kinetics of phosphorylation of the receptor in transfected cells or T47D is slow with maximal phosphorylation observed after 90 minutes. The reason for this slow phosphorylation of the receptor remains unclear. To understand the reasons for slow phosphorylation of the receptor we examined several cell lines under different conditions. We confirm that endogenous DDR1 is stimulated slowly by collagen in T47D cells when they are adherent but detaching them results in rapid phosphorylation (within 10 minutes) of DDR1. This was further confirmed using a semi-adherent (colo201) and a suspension (K562) cell line expressing endogenous DDR1. However, in the adherent fraction of colo201 cells there was no detectable phosphorylation of DDR1. Plating K562 on fibronectin to mimic adherent conditions altered the kinetics of phosphorylation from rapid to slow, similar to adherent cells. The slow kinetics of phosphorylation in the adherent state was not due to cell-cell contact as EDTA, a chelating agent had no major effect. However, pervanadate was able to induce DDR1 phosphorylation indicating that a phosphatase may inhibit or delay the phosphorylation on DDR1. Collagen stimulation of cells in either adherent or suspension conditions does not increase ERK phosphorylation. However, DDR1 in either adherent or suspension conditions associates with p85α subunit of PI-3 kinase. In addition, as an alternative approach, we have stably expressed a chimeric TrkA/DDR1 receptor in NIH3T3 cells, to study the signalling properties of DDR1. We show that the chimeric receptor does dimerize upon stimulation with NGF but it phosphorylates poorly upon stimulation. This suggests that there may be inhibitory constraints on the phosphorylation of the intracellular domain of DDR1. This is the first receptor tyrosine kinase whose kinetics of phosphorylation are dependent on cellular context. The interaction of the cells with the matrix, rather than cell-cell contact is probably responsible for the inhibition in phosphorylation.

CELL AND TUMOR BIOLOGY 46: Models and Imaging of Tumor Progression and Metastasis

#5224 MRI-detected heterogeneity of water diffusion in orthotopically implanted murine renal carcinoma. P. N. Venkatasubramanian, Gerald Soff, Esther French, Brian Tom, and Alice M. Wyrwicz. *Center for M.R. Research, Evanston, IL, and Northwestern University, Chicago, IL.*

Although studies have suggested the existence of two compartments for water diffusion in several tissue types including tumors, the exact nature of this compartmentation is not understood. MR-measured diffusion parameters can provide an insight into such cellular organization. Depending on the rate of exchange between compartments, signal in diffusion weighted MR images will exhibit mono- or bi-exponential rate of decay with increasing gradient strength. Traditionally, diffusion MR studies have observed only the fast diffusing component, since the high *b* values necessary for detecting the slow component cannot be generated readily on clinical and most research imaging systems with limited gradient strength. We used a microimager fitted with gradients of high strength to investigate diffusion of water in murine renal carcinoma, and found the existence of both mono- and bi-exponential decay in a regionally specific pattern. 1×10^5

THE THREE SPECIFIC AIMS OF THE PROJECT ARE:

- *Specific Aim 1:* transfect normal prostatic epithelium with the E6 and E7 genes of HPV16 to produce immortalized cell lines, then genotype these cell lines to determine 8p and 8q status: retention of 8p sequences, loss of 8p sequences, or loss of 8p+gain of 8q sequences [iso(8q)];

- *Specific Aim 2:* determine whether loss of 8p sequences or loss of 8p+gain of 8q sequences is associated with expression of the transformed or invasive/metastatic phenotypes in the E6/E7 immortalized cells;

- *Specific Aim 3 (Long Term Goals):* isolate 8p-specific and 8q-specific genes that contribute to the transformed or invasive/metastatic phenotype in E6/E7 immortalized cells.

During 2001, Dr. Kant continued to learn basic cancer biology and the techniques used for the project. He also attended weekly journal club meetings organized by Dr. Macoska, seminars in cancer biology organized by Hematology/Oncology division, the comprehensive cancer center, and other various departments of the University. Dr. Kant presented his research to members of the Genito-Urinary Oncology Program of the University of Michigan Comprehensive Cancer Center and to members of the University of Michigan Prostate SPORE.

PROGRESS ON SPECIFIC AIM 1:
Dr. Kant created a total of 6 epithelial and 3 fibroblastic cell lines derived from explanted human prostate tissues immortalized using a recombinant replication deficient retrovirus carrying E6 and E7 genes of HPV-16. Five of the six epithelial and one of the three fibroblastic cell lines have been karyotyped to date by the laboratory of our collaborator, Dr. Jeremy Squires, at the University of Toronto. Utilizing spectral karyotyping (SKY) analysis, the composite karyotypes of these cell lines have been determined and are shown below (the number in brackers refers to the number of metaphases that were individually karyotyped for each cell line):

Human Prostate Epithelial Cell Lines (changes involving chromosome 8 are **bold):**

N10B1 p26 [6]
44-48,XY,-9,-14,+20]

N12B3 p22 [10]
52-58,XY,+6,+7,**+8**,+9,+11,+del(16)(p12),+der(17)t(1;17)(q22;p13.3),+19,+20x2

N15C6 p20 [10]
42-44, X, der(Y)(dupinv)(q11q12), **der(8)t(8;19)(q10;p10), +der(8)t(8;20)(p10;q10)x2**, -19, -21, -21
(this cell line has one normal 8, one 8q, and two 8p fragments)

N17A3 p27 [9]
34-48,XY, -11, -19, +20, -22 der(15)t(11;15)(q14;q24) [8]
87,X,X,Y,Y [1], +20x3 [1], der(15)t(11;15)(q14;q24) [1]

N33B2 p26 [7]
40-44XY, -19, -22, der(1)t(1;13)(p36;q32), **iso(8q),** der(13)t(11;13)(p10;q10), der(15)t(15;19)(q10;p10) [7]
85-87 XXYY, der(1)t(1;13)(p36;q32), **iso(8q)** der(13)t(11;13)(q10;q10), der(15)t(15;19)(q10;p10) [2]

N1 p40 [10]
46, XY

Alterations Involving Chromosome 8
Clonal alterations involving chromosome 8 were observed in three of the five epithelial cell lines and a non-clonal alteration was observed in a fourth cell line. The clonal alterations included trisomy 8 (N12B3), der(8)t(8;19)(q10;p10),+der(8)t(8;20)(p10;q10)x2 (N15C6) and iso(8q) (N33B2). The non-clonal alteration comprised a dicentric chromosome consisting of part of chromosome 3 and the p-terminal region of chromosome 8 in N10B1 cells.

Array comparative genomic hybridization (aCGH) experiments performed in collaboration with Dr. Colin Collins at the University of California, San Francisco revealed that chromosome 8 alterations continue to evolve over time. At low passage (p20), spectral karyotype analysis N15C6 cells demonstrate complex alterations involving chromosome 8 (see above). At a similar low passage number (p26), aCGH analysis demonstrates that there is no net gain or loss of chromosome 8 genetic information despite these complex translocation events in N15C6 cells. However, higher passage N15C6 cells (p45) demonstrate loss of one copy of 8p by aCGH (**Figure 1**). ***These results show that complex structural alterations leading to loss of the 8p chromosomal region occur during immortalization and early transformation in prostate epithelial cells.***

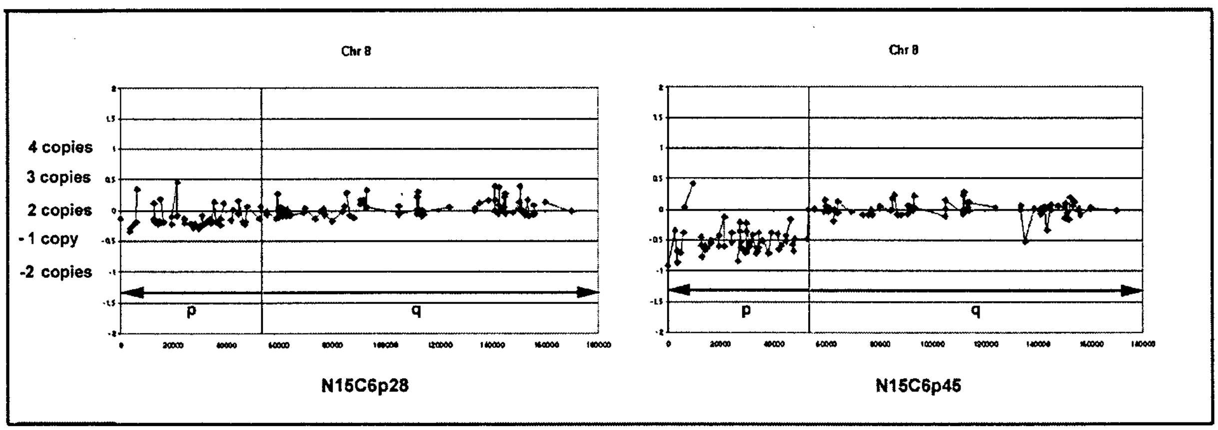


Figure 1. Array CGH of N15C6 Low (p28) and High (p45) Cells. Two copies of chromosome 8 are evident in low passage cells (left) while loss of one copy of 8p is evident in high passage (right) cells.

PROGRESS ON SPECIFIC AIM 2:

Comparison of the malignant potential of low and high passage N15C6 cells showed that high passage cells demonstrated an augmented ability to form colonies in soft agar compared to low passage cells (**Figure 2**). Changes in the genetic composition of high passage and low passage N15C6 cells were limited to gain of 5p, loss of 8p and loss of 11p. Therefore, at least some of the augmentation in expression of the malignant phenotype evident in high passage N15C6 cells may be attributable to loss of sequences mapping to 8p.

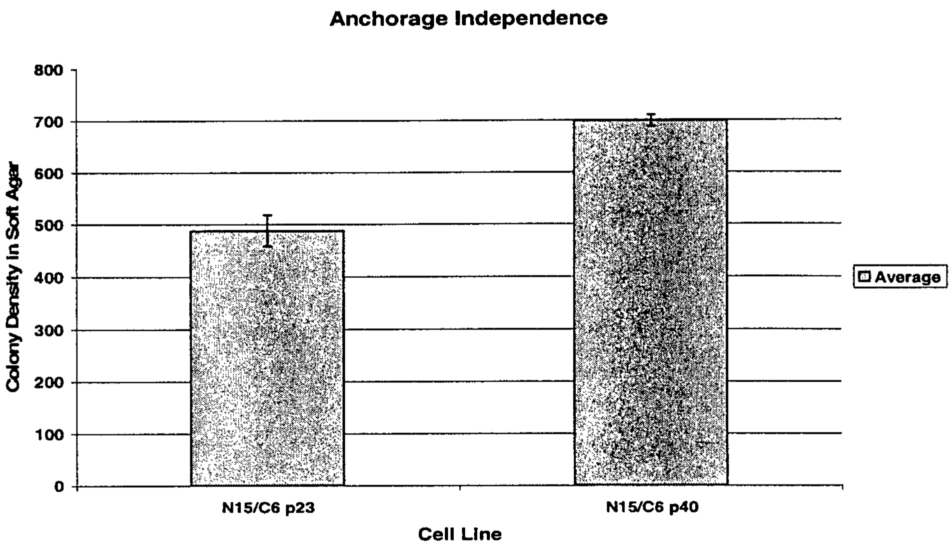


Figure 2. Anchorage Independence of Low vs. High Passage N15C6 Cells. High passage N15C6 cells were able to form colonies in soft agar at an efficiency 1.5X that of low passage N15C6 cells.

PROGRESS ON SPECIFIC AIM 3 (LONG TERM GOAL):

aCGH Clone	Log2Ratio	GenBank	Position	CytoBand	Gene
RP11-117P11	-0.8606	G11277	1320842	8p23.3	MYOM2
RP11-246G24	-0.6757	H72324	2285417	8p23.2	predicted
RP11-121F7	-0.7197	Z50929	3173675	8p23.2	CSMD1
RP11-235O5	-0.5841	Z23725	10669100	8p23.1	SOX7; PINX1
RP11-262B15	-0.5956	G13875	10230290	8p23.1	MSRA
RP11-112G9	-0.7808	Z24056	10398938	8p23.1	MSRA
RP11-252K12	-0.6179	Z24258	10994273	8p23.1	predicted
RP11-254E10	-0.6674	G17225	11239562	8p23.1	predicted
RP11-235I5	-0.6449	T72650	11829647	8p23.1	predicted
RP11-31B7	-0.5031	Z24368	12247145	8p22	predicted
RP11-92C1	-0.5505	L18702.1	12378079	8p22	predicted
RP11-182G2	-0.6087	Z24238	14564896	8p22	N33
RP11-233H21	-0.5403	H41214	19458127	8p21.3	FLJ10569
RP11-110I16	-0.8417	U97145.1	21707690	8p21.3	GFRA2
RP11-158F9	-0.7046	Z52979	23804356	8p21.2	predicted
RP11-232J22	-0.5984	AB020690	25331832	8p21.2	PNMA2
RP11-199N14	-0.6076	AF067396.1	25212086	8p21.2	BNIP3L
RP11-116F9	-0.7317	T59517	27367583	8p21.1	predicted
RP11-204M16	-0.6299	AB002390	29970105	8p12	LYSAL1
RP11-204M16	-0.5352	AB002390	29970105	8p12	LYSAL1
RP11-288N10	-0.6467	R01197	30106037	8p12	LOC51312
RP11-275K7	-0.6343	T81337	28078768	8p12	FLJ21616
RP11-277I21	-0.6864	G17229	28015457	8p12	FLJ21616; KIF13B
RP11-5J20	-0.6212	R05981	31642386	8p12	predicted
RP11-287N19	-0.5016	S67861.1	28509093	8p12	GTF2E2
CTD-2020E14	-0.5160	WRN	29455999	8p12	WRN
RP11-57I3	-0.7205	Z16888	32974735	8p12	NRG1
RP11-122D17	-0.7229	AF009227.1	32974735	8p12	NRG1
RP11-237M13	-0.6566	G17257	36471177	8p12	KIAA1777
RP11-210F15	-0.5935	Z51338	37222391	8p12	none
RP11-265K5	-0.5154	R55295	38897318	8p12	predicted
RP11-48D21	-0.6795	G17240	41111144	8p11.21	predicted
RP11-107P5	-0.6160	SHGC-35410	nd	nd	nd
RP11-76B12	-0.6710	X15215	nd	nd	nd

Figure 3. Detail of 8p Sequence Deletions in High Passage N15C6 Cells. aCGH clone = identify of clone spotted onto array CGH slide; Log2Ratio = fold difference in dosage from low passage cells (-0.5 = 1 copy); GenBank = associated accession number; Position = physical location on chromosome 8 (from http://genome.ucsc.edu); CytoBand = cytogenetic location of aCGH clone; Gene = associated known or predicted gene sequence at that locus.

We are now in the process of identifying genes mapping to 8p that are down-regulated in high passage compared to low passage N15C6 cells. Some candidate genes can be inferred from the table shown in **Figure 3**, which identifies several known and predicted genes mapping to 8p that are deleted for one copy in high passage N15C6 cells. These sequences may comprise candidate tumor suppressor genes as one copy is deleted and the other may be inactivated through deletion or mutation. These candidate tumor suppressor gene sequences will be utilized in complementation experiments and transfected back into high passage N15C6 cells to see if their expression reverts or reduces expression of the malignant phenotype (measured by reducations in proliferative rate, anchorage independence, etc.).

ONGOING WORK

Our laboratory has just begun characterizing the cell lines developed from this project. We expect that several publications and external funding will result from these studies, and recently submitted the first manuscript for publication (see below).

APPENDIX

1) Key Research Accomplishments

- 6 epithelial and 3 fibroblastic cell lines were developed from explanted human prostate tissue
- 5/6 epithelial and 1/3 fibroblastic cell lines were genotypically characterized using spectral karyotyping (SKY)
- 3/5 epithelial cell lines demonstrated clonal numerical or structural alterations of chromosome 8, including:
    - trisomy 8 (N12B3)
    - der(8)t(8;19)(q10;p10),+der(8)t(8;20)(p10;q10)x2 (N15C6)
    - iso(8q) (N33B2).
- The N15C6 cell line continued to evolve in culture and demonstrated loss of 8p sequences at high passage
- High passage N15C6 cells also exhibited augmented expression of the malignant phenotype, suggesting that loss of 8p sequences may be associated with prostate tumor initiation and progression.
- Several genes and predicted proteins have been identified mapping to the deleted 8p region that can be investigated as candidate tumor suppressor genes

2) Reportable Outcomes

PRESENTATION AND PUBLICATIONS

Extramural Presentations:

American Association for Cancer Research annual meeting, April 6-10, 2002, San Francisco, CA. Title: Paracrine Interactions Modulate Phenotypic and Genotypic Expression In Transformed Prostatic Epithelial Cells. R. Kant, E.K. Cockrell, M.A. Rubin[3], B. Beheshti, J.A. Squire, A. Powell, J. Rhim and J.A. Macoska.

Intramural Presentation:

Cancer Genetics Program, The University of Michigan Comprehensive Cancer Center, May 17th 2002. Topic: Paracrine interactions modulate phenotypic and genotypic expression in transformed prostatic epithelial cells.

Cancer Biology Research Seminar, The University of Michigan Comprehensive Cancer Center, June 11th 2002. Topic: Paracrine interactions modulate phenotypic and genotypic expression in transformed prostatic epithelial cells.

Publications (submitted)

Kant R, Chaib H, Zalewski C, Ethier SP, Rubin MA, Washburn JG, MacDonald JW, Beheshti B, Powell A, Rhim J, Squire JA and Macoska JA. Paracrine Interactions Define the Malignant Potential of Human Primary Prostate Tumor Epithelial Cells.

CELL LINES: see report text for list

FUNDING: An R01 application is in preparation in response to PA-02-116, "Age-Related Prostate Growth: Biologic Mechanisms", issued June 25, 2002. This PA specifically states that "The NCI has a special interest in receiving applications that address the role of aging tissue microenvironment (stromal cells) in prostate carcinogenesis and/or progression." We intend to utilize our unique cell lines as model systems in response to this program announcement.

3) Copies of the AACR abstract and the manuscript preprint are attached to this report.